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VALORIZATION OF SOLID WASTES FROM THE BREWERY AND BIODIESEL INDUSTRIES FOR THE BIOPRODUCTION OF NATURAL DYES

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Abstract - This study aims to assess a new approach for concomitant valorization of two industrial wastes - raw glycerin and spent brewer's yeast - for the bioproduction of valuable carotenoids. Microbial pigments have numerous applications in the food and cosmetic industries. First, four cultures of yeasts were screened using pure glycerol and either $(NH_4)_2SO_4$ or urea as carbon and nitrogen sources, respectively. The ability of the best performing culture to accumulate carotenoids was investigated in a medium in which only wastes were supplemented as carbon and nitrogen sources. All the fermentations were carried out in 500 mL-Erlenmeyer flasks containing 150 mL of the medium. Microbial culture was incubated at 30 °C and 150 rpm for 120 h. Particularly, *R. marina* was the strain with the biggest potential to produce total carotenoids (up to 420 μg g^{-1}). The four major carotenoid pigments identified by LCAPCI-MS were β-carotene, γ-carotene, torulene, and torularhodin. All fermentation assays and all the determinations were performed in triplicate. Results show that the bioprocess proposed in this work is technically and environmentally feasible, and sustainable. The simultaneous use of raw glycerin and spent brewer's yeast for the production of carotenoids by *R. marina* is reported for the first time.
Keywords: Production of natural dyes; β-carotene; Valorization of solid wastes.

INTRODUCTION

Increasing ecological awareness, globalization of markets and management costs make industrial solid waste a relevant and contemporary issue (Mbuligwe and Kaseva, 2006). Thus, the use of solid waste (low-cost materials) aims to save raw materials while generating competitive advantages and economic opportunities (Vandermeersch et al., 2014).

Raw glycerin, still called industrial or crude glycerin, is an important by-product of the biodiesel industry (Caldeira et al., 2016). Due to commercial restrictions of the market or the purification processes, often the raw glycerin is discarded as solid residue (Oliveira et al., 2016). When this occurs, it is usually incinerated and co-processed in cement kilns, which increase the overall biodiesel production cost.

The brewing industry uses a substantial amount of water and generates a large amount of solid waste. The filtration of malt, separation of yeast from fermented medium, and treatment of industrial effluents are the largest generators of solid wastes (Mathias et al., 2014; Fakova and Van der Poll, 2013; Fillaudeau et al., 2006; Kanagachandran and Jayaratne, 2006; Thomas and Rahman, 2006). Spent brewer's yeast (SBY), which is also referred to as spent slurry, may be a by-product of the process and can be marketed to the animal feed industry. However, the surplus or non-marketed part is solid waste or mixed with the liquid effluent, increasing the organic load of the treatment plants. The

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spent brewer's yeast is a key residue not only because of the high amount generated, but also because of its poor biological stability, high water content, and high level of enzymatic activity, which make it difficult to store. The main destinations of spent brewer's yeast are composting, effluent disposal, biodigestion, and landfill disposal (Goldammer, 2008; Fillaudeaua et al., 2006).

Carotenoids (a kind of natural dye) are high value-added compounds with potential application mainly in the food industry as natural dyes to replace normally used chemically synthesized dyes, whose assimilation by humans can be harmful to health. Moreover, carotenoids have human health benefits as precursors of vitamin A and have antioxidant, anticancer, and immune response properties (Abad and Turon, 2012).

Nowadays, most of the terpenoid pigments used industrially are obtained by chemical synthesis (Mezzomo and Ferreira, 2016). However, seasonal and geographic variability, hazardous wastes generation beyond other environmental and sustainability considerations have sparked scientific interest in the microbial production of carotenoids (Mata-Gómez et al., 2014). In this direction, many species of the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces*, *Cystofilobasidium*, *Xanthophyllomonas* and *Phaffia* have been reported as profitable biocatalyst choices for natural dyes production (Silva et al., 2016; Mata-Gómez et al., 2014; Frengova and Beshkova, 2009). Microbial production of carotenoids can be performed using many types of substrates, like glucose, fructose, lactose, alcohols, and organic acids, which can be provided by lower-cost raw materials such as cane juice, molasses, and whey (Marova et al, 2012; Aksu and Eren, 2007; Valduga et al., 2007). It is important to highlight that few works have dealt with the valorization of raw glycerin, although some studies have focused on using glycerol as the main carbon source (Chatzifragkou and Papanikolaou, 2012). Meanwhile most research articles cover high-cost sources of nutrients (Cardoso et al., 2016; Saenge et al., 2011; Mantzouridou et al., 2008).

Nutrients and vitamins are essential in the production medium for microbial growth and carotenoid production (Aksu and Eren, 2007) and comprise a significant portion of the costs to prepare fermentation media, which may limit the economic viability of the process. Besides, it is important to emphasize along with the substrate that the production rate of pigments by many microorganisms may be a relevant limitation for their industrial application (Moliné et al., 2012).

Thus, the object of the present work is to evaluate strategies that improve sustainability and manage resource supply in the production of products with high added value. In this context, the feasibility of replacing primary resources with two internationally produced solid wastes, raw glycerin from biodiesel production and spent yeast from the brewing industry, was investigated in the production of natural dyes by *Rhodotorula* strains, which are native to Brazil. To our best understanding, carotenoid production by fermentation of these two solid wastes as sole nutrient sources has not been explored yet.

MATERIALS AND METHODS

Sampling, classification, and characterization of solid wastes

A 20 L aliquot of raw glycerol (which was to be discarded as solid waste) was collected from a biodiesel plant located in the State of São Paulo, Brazil. After vigorous mixing, three kilograms of solid waste were sub-sampled (USEPA, 1995) in a laminar flow chamber into polypropylene vials and stored at 4 °C prior to analysis and fermentation tests.

The SBY was collected from a brewing industry, located in the State of Rio de Janeiro, Brazil. A plastic bag containing 20 kg of solid waste was received and the samples were obtained using a stainless-steel grain sampler according to the procedures described by USEPA, (1980). Three kilograms of solid waste were sub-sampled by a cone and quartering procedure (USEPA, 1995) in a laminar flow chamber using a sterile spoon and stored in sterile flakes before testing and chemical analysis.

Samples of both solid wastes were also submitted to waste classification procedures according to Brazilian Standard 10004 (2004), which tests for flammability, corrosivity, and reactivity. In addition, a toxicity characteristic leaching procedure was performed (Title 40 of the United States Code of Federal Regulations and USEPA (2007).

The water content in the samples of industrial glycerin was determined by Karl Fischer coulometric titration (ASTM, 2007). The water content in SBY samples was determined using an infrared moisture analyzer. pH values of solid wastes were measured in suspensions of distilled water 1: 1 (p p^{-1}), according to Brazilian Standard 10004 (2004). The concentrations of total nitrogen and total phosphorus were determined by colorimetry, according to the methodologies USEPA 365.2 and USEPA 353.2, respectively (USEPA, 2007).

Aliquots of 1g of each sample of industrial waste were subjected to superior calorific value (SCV) quantifications. The determinations of SCV were performed using an Ika C200 bomb calorimeter according to the procedures preconized by method DIN 51900-1 (2000).

The concentrations of cyanides and sulfides were also determined by cyanide ion-selective electrode and colorimetry, respectively (APHA, 1992). The

metals were determined by using inductively coupled plasma mass spectrometry, according to the methods described in USEP 6020 and USEPA 7474 (USEPA, 2007). To quantify the chemical elements, the samples were previously digested in a microwave oven using both nitric acid and hydrogen peroxide, according to the methodology described in USEPA 3030B (USEPA, 2007).

The concentration of glycerol in industrial glycerin samples was determined by potentiometric titration (ASTM, 2010). Methanol was quantified in the raw glycerin samples using gas chromatography, flame ionization, and head-space sampling, according to the methods USEPA 8260B and 5021A, respectively (USEPA, 2007).

Selection of *Rhodotorula* strain for carotenoids production

The carotenoid production tests used four strains of *Rhodotorula* (*R. pallida, R. minuta, R. mucilaginosa, and R. marina*), all isolated in Brazil and kindly provided by the Strain Bank of the School of Chemistry at the Federal University of Rio de Janeiro. Pure glycerol was used as source of carbon and energy (20 g L^{-1}) in the culture selection studies. All assays were performed at 150 rpm in 500-mL Erlenmeyer flasks containing 150 mL of liquid medium (g L^{-1}): $(NH_4)_2SO_4$, 5.0; KH_2PO_4 5.5; Na_2HPO_4 3.3; $MgSO_4.7H_2O$ 0.5; $MnSO_4.7H_2O$ 0.2. The pH of the culture medium was adjusted to six using 1 N NaOH solution and autoclaved at 121 °C for 20 min.

Experiments for solid waste recovery

Subsequent tests were carried out following microbial selection tests to evaluate the feasibility of replacing primary sources of carbon and nutrients by low cost products and/or solid waste. Thus, in some experiments, ammonium sulfate (5 g L^{-1}) in the liquid medium was replaced by other low-cost nitrogen sources: commercial urea (2 g L^{-1}) and SBY (1 g L^{-1}). In addition, tests substituted pure glycerol (10 g L^{-1}) with raw glycerin (10 g L^{-1}), which was to be discarded as solid waste.

Aliquots of the fermented medium were centrifuged at 5000 rpm for 10 min at 4 °C to separate the biomass. Then, the biomass was resuspended in petroleum ether and ruptured with the aid of a bead grinder. Then the carotenoids were extracted with petroleum ether. The suspension was centrifuged and the supernatant collected. Fresh petroleum ether was mixed into the pellet and centrifuged until the color disappeared. The petroleum ether extracts were collected, and the absorbance was determined at 455 nm. The total carotenoids content was determined spectrophotometrically in which β-carotene (Sigma, USA) was used as the standard.

The different carotenoids were identified using liquid chromatography (HPLC), Agilent 1260 Infinity, coupled with a high-resolution Agilent 6420 mass spectrometer (MS). The chromatograph was equipped with a C18 column (Agilent and Poroshell 120 SB-C18, 75 mm × 2.1 mm, 2.7 μm, 120 Å) and an atmospheric pressure chemical ionization (APCI) interface. N_2 was used as the nebulizer gas under the following conditions: 350 °C, 7 L min^{-1}, 40 psi. Aliquots of 20 μL were eluted with a water and acetonitrile mixture (7:3 v v^{-1}) at a flow rate of 1 mL min^{-1} in isocratic mode. Column temperature was controlled at 40 °C. The mass spectra of carotenoids were acquired in positive ion and full scan mode of m/z 50-700 with shredder at 250 V, and acceleration cell at 7 V. Simultaneously, the diode array detector (DAD) detector recorded UV spectra in the range from 200 nm to 600 nm. The four identified carotenoids were quantified using the same chromatographic system previously described and external standardization (Nan et al., 1988).

The results for the average production of carotenoids obtained from tests that substituted primary sources of carbon and nutrients for low-cost supplies and/or solid wastewere submitted to ANOVA and randomized multiple comparison of means (Tukey's test) at the significance level of 5%. In all cases, the analyses were performed with the aid of the statistical software Statistica, version 5.5 (StatsoftInc). All quantitative determinations were performed in triplicate (n = 3). Results are presented as mean and standard deviation ($p > 0.05$).

RESULTS AND DISCUSSION

Classification and characterization of the studied solid wastes

The physical-chemical characterization data of the two solid wastes: SBY and raw glycerin, from the brewing industry and biodiesel production, respectively, are shown in Table 1. Flammability, corrosivity, and reactivity characteristics were not found in samples of either waste investigated (Table 1). Sodium and potassium were the most abundant metals in the raw glycerin samples and are related to the catalysts used in the biodiesel production process (potassium hydroxide or sodium methoxide). In the SBY, these elements were nutrients (Yenush, 2016). Both solid wastes contained elements such as iron, manganese, and aluminum, which may be related to incorporation of corrosion products from the metal surfaces of industrial equipment and pipes. Both solid wastes also had other heavy metals in very low concentrations, which were consistent with the materials and processes employed in the industries. Unlike raw glycerin, the SBY samples showed high concentrations of total nitrogen, confirming the organic

Table 1. Characterization of the solid wastes used in the raw material substitution tests for carotenoids production.

Determination	Raw Glycerin	Spent Brewer's Yeast (SBY)
Water content (%)	13 ± 2	8 ± 1
Solid content (%)	< 0.5	> 70
pH (1:1 in water)	5.3 ± 0.1	5.7 ± 0.2
Sulfide (mg of H_2S kg^{-1})	4 ± 1	5 ± 1
Cyanide (mg of HCN kg^{-1})	45 ± 1	34 ± 4
Flammability	not flammable	not flammable
Corrosiveness (mm $year^{-1}$)	0.63 ± 0.03	0.70 ± 0.08
SCV (cal g^{-1})	3060 ± 180	3921 ± 311
Total aluminum (mg kg^{-1})	708.9 ± 103.7	61.4 ± 43.0
Total arsenic (mg kg^{-1})	10.8 ± 2.2	1.5 ± 1.0
Total barium (mg kg^{-1})	38.9 ± 10	4.4 ± 1.7
Total cadmium (mg kg^{-1})	1,0 ± 0,1	0,5 ± 0,4
Total calcium (mg kg^{-1})	9791 ± 1172	1276 ± 294
Total chromium (mg kg^{-1})	459 ± 183	377 ± 265
Total iron (mg kg^{-1})	7824 ± 271	1022 ± 572
Total potassium (mg kg^{-1})	20584 ± 1275	47734 ± 9336
Total nickel (mg kg^{-1})	37.7 ± 5.3	4.9 ± 1.4
Total manganese (mg kg^{-1})	34.8 ± 3.7	58.1 ± 41.3
Total lead (mg kg^{-1})	8.1 ± 1.8	1.1 ± 0.8
Total selenium (mg kg^{-1})	7.0 ± 0.4	0.4 ± 0.2
Total zinc (mg kg^{-1})	<1.32	168.7 ± 39.8
Total sodium (mg kg^{-1})	29444 ± 288	307 ± 6
Total mercury (mg kg^{-1})	<19	<19
Nitrogen (%)	< 0.5	4.3 ± 0.5
Glycerol (%)	82 ± 4	< 0.1
Methanol (%)	0.5 ± 0.1	< 0.1

nature of the studied materials. Nitrogen is a natural component of living organisms, indicating a presence of amino acids, peptides, and various vitamins in their cellular tissues (Madigan et al., 2008; Mathias et al., 2014).

In both solid wastes, ethane and chlorinated ethenes, chloroform, benzene, benzo(a)pyrene, chlorobenzene, Aldrin, Endrin, and several other organic compounds listed in Annex F of Brazilian Standard 10.004 were detected in concentrations lower than their limits of quantification (LOQ) in the leachate extracts. In all cases, LOQ were less than 0.0015 mg L^{-1}.

The TCLP results (Table 2) of all the solid wastes samples indicated that the heavy metals present in the samples are retained in the solid fraction. The chemical analyses classify both wastes as non-hazardous and non-inert (Class 2A).

Selection of *Rhodotorula* strain for carotenoids production

Figures 1 A-C report the growth profile, substrate consumption, and pH variation, respectively, for cultures of the four *Rhodotorula* strains studied (*R. pallida, R. marina, R. minuta,*and *R. mucilaginosa*) in culture medium containing glycerol as the main carbon source. All microbial strains were able to grow at the expense of glycerol in mineral medium with similar

Table 2. Leachate concentration of the wastes (spent brewer's yeast and raw glycerin) - TCLP.

Parameter (mg L^{-1})	Raw Glycerin	Spent Brewer's Yeast (SBY)	MPV*
Total arsenic	0.05	0.04	1.0
Total barium	1.73	0.05	70.0
Total cadmium	< 0.004	< 0.004	0.5
Total lead	< 0.009	< 0.009	1.0
Total chromium	< 0.010	< 0.010	5.0
Total fluoride	< 0.150	< 0.150	150
Total mercury	< 0.001	< 0.001	0.1
Total silver	< 0.005	< 0.005	5
Total selenium	< 0.009	< 0.009	1.0

*MPV is maximum permitted value.

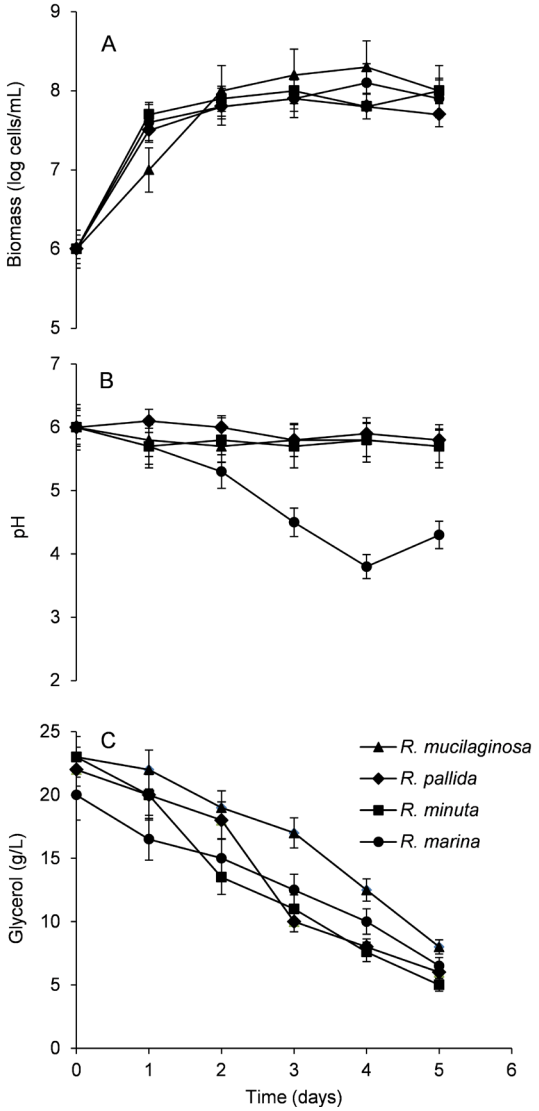

Figure 1. Cultivation of *Rhodotorula pallida, Rhodotorula marina, Rhodotorula minuta,* and *Rhodotorula mucilaginosa* strains in liquid medium containing pure glycerol as carbon source. (A) growth profile,(B) pH variation of medium in fermentation, and (C) glycerol consumption.The bars indicate the standard deviation of the mean.

growth, yielding the maximum cell concentration, about 10^8 cells mL^{-1}, in 48 h of culture (Fig 1A). The exception was *R. marina* that reached the maximum value of cellular concentration in 72 h (Fig. 1A).

The pH value of the reaction medium remained practically constant throughout the monitored time for the cultures *of R. pallida, R. minuta,* and *R. mucilaginosa* (Fig. 1B). In the case of *R. marina*, the pH decreased to values close to 4. The glycerol concentration profile in the fermented media was similar in all cultures. Under the nutritional conditions tested, total consumption of glycerol was not detected for any of the strains, and ranged from 48% to 78% (Figure 1C). The percentage of glycerol consumption in the exponential phase of microbial growth was quite different, usually representing a value less than 50% of the content consumed throughout the process.

For all microbial cultures, the production of total carotenoids increased after 2 days of inoculation (Figure 2), notwithstanding that the synthesis profiles were different. Except for *R. pallid*a, total carotenoids produced by other species reached the maximum values at the end of process (5 days), i.e., between 40 and 420 μg g^{-1} of cell. In fact, the cultivation of *R. pallida* on glycerol containing medium resulted in the highest total carotenoid concentration (87 μg g^{-1} of cell) on the third day. The decrease of concentration of total carotenoids over a more extended time was already reported by Varmira et al (2016) and Varzakakou et al. (2011) for the growth of fungi in bioreactors. According to these authors the decrease in pigment concentration with time is due to cell membrane autolysis and chemical oxidation reactions of pigments.

The production of carotenoids from glycerol was not a function of the species used, because the four microbial cultures showed similar growth. The analysis of Figure 2 also emphasizes the potential of *R. marina* and *R. minuta*strains in the production of carotenoids, 420 and 310 μg g^{-1} of dry cells, in five days of process,

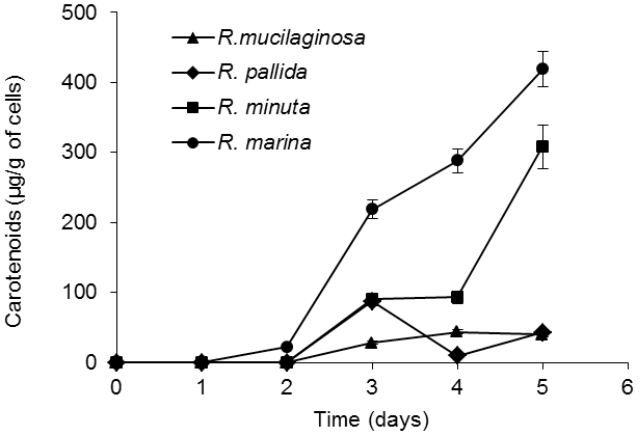

Figure 2. Production of carotenoids by *Rhodotorula* spp grown in pure glycerol supplemented medium. The bars indicate the standard deviation of the mean.

evidencing the importance of the step of selecting the microbial strain.

Several studies have shown that the productivity of carotenoid synthesis is influenced by the yeast used. Shih and Hang (1996) reported that cultivation of *R. rubra* NRRL-15596 in brine from cabbage fermentation led to a production of 130 μg g^{-1} of pigments. Squina et al. (2002), working with a strain of *R. rubra*, evaluated the growth and production of carotenoids in different concentrations of sugarcane juice supplemented with yeast extract, with or without peptone or inorganic salts solution. The concentration of total carotenoids ranged from 60.0 to 426.6 μg g^{-1} dry mass, depending on the composition of the medium. These authors also found that, for the same nutritional conditions, the maximum yield of total carotenoids for *R. rubra* cultivars was much higher than for the cultures with *R. glutinis* (197 μg g^{-1}). Studies using different strains of *R. minuta* obtained total carotenoid contents varying from 40 to 100 μg g^{-1}(Peterson et al., 1958; Perrier et al., 1995; Squina and Mercadante, 2003). Squina and Mercadante (2003) determined differences in the concentration of total carotenoids in dry cells of *R. glutinis* (251.7 μg g^{-1}), *R. rubra* (123.5 μg g^{-1}), *R. araucariae* (11.2 μg g^{-1}), *R. lactosa* (105.8 μg g^{-1}), and *R. minuta* (103.7 μg g^{-1}) on day 5 of culture, using medium containing glucose as substrate (28 °C, 150 rpm, and exposure to intense lighting using a fluorescent lamp). Thus, the potential of the *R. marina* strain in the production of these high added value products is demonstrated and justified the subsequent trials to reduce the overall cost of the process by replacing expensive carbon and less expensive nutrient sources with industrial waste.

Assessing low-cost materials as carbon and nitrogen sources for production of carotenoids by *R. marina*

Figure 3 shows the results of carotenoid concentrations for the second day of fermentation by *R. marina*, as only after this period could the biosynthesis be detected, irrespective of the three N sources studied. The substitution of pure glycerol by raw glycerin (10 g L^{-1} of glycerol) had very little influence on the quantity of carotenoids produced when urea or SBY was used as a source of macro-nutrients. This result can be related to the composition of SBY, which contains high concentrations of proteins (source of N) and B vitamins, favoring the cellular metabolism.

Aiming to assess the potential of biodiesel-derived glycerol (raw glycerin) and spent brewer's yeast as feedstocks for carotenoids bioproduction, the productivity was monitored at 24-h intervals over a 5-day period (Fig. 4); whilst seeking to increase sustainability of the process. Besides, such low-cost substrates could greatly increase the economic cost-effectiveness of carotenoids production and enable biodegradation of problematic industrial wastes. From

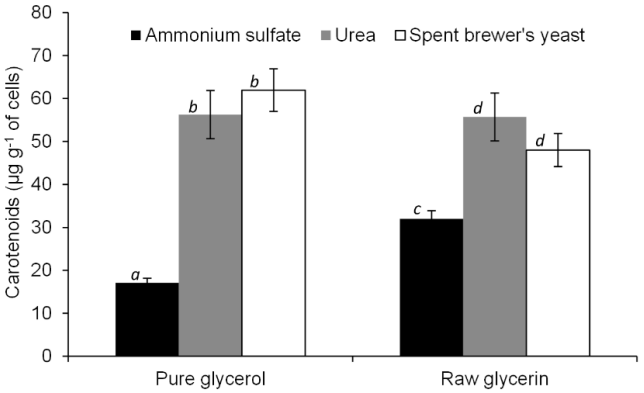

Figure 3. Production of carotenoids on the second day of the process for *R. marina* cultivation using pure glycerol or raw glycerin as primary carbon source and ammonium sulfate, urea, and spent brewer's yeast as nutrient source. For the same carbon source, the same lowercase letters indicate that the data are not significantly different, according to the Tukey test (p> 0.05). The bars indicate the standard deviation of the mean.

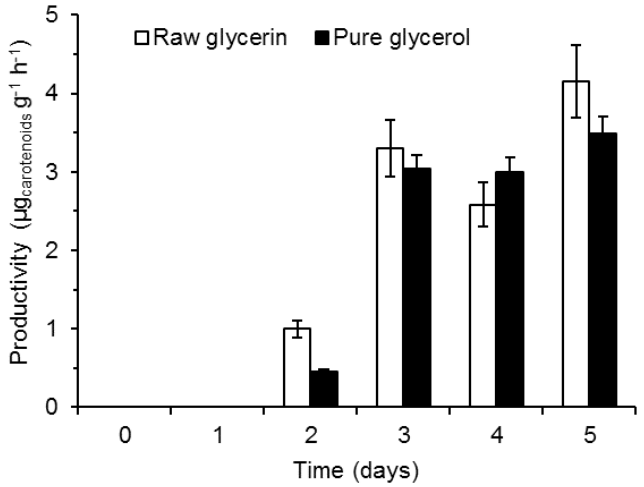

Figure 4. Production of carotenoids by *R. marina* using raw glycerin or pure glycerol as primary carbon source and spent brewer's yeast as nitrogen source. The bars indicate the standard deviation of the mean.

the third day of the bioprocess, a slight influence of the time on the carotenoid production can be noted, irrespective of the type of carbon source, by using the SBY mainly as source of nitrogen.

Literature reports great variation among specific carotenoid production rates, from 6 up to about 152 $\mu g_{carotenoids} g^{-1}$ day^{-1}, depending on yeast strain, process condition and substrate availability (Table 3). However, most research articles cover high-cost sources of nutrients. Thus, the comparison of data recorded in Table 3 with Figure 4 clearly demonstrates the technical feasibility of using both raw glycerol and SBY as C and N sources (low-cost materials), respectively, for carotenoids production by the *R. marina* strain.

Chromatographic analyses indicated that *R. marina* produced four main pigments (β-carotene, γ-carotene, torularhodin, and torulene - Table 4), regardless of whether the culture was made in liquid medium containing raw glycerin and any of the two low-cost nutrient sources (Urea and SBY). At the second day of the bioprocess, β-carotene was the main carotenoid (25 µg g^{-1}), followed by torularhodin (15 µg g^{-1}), torulene (12 µg g^{-1}), and γ-carotene (4 µg g^{-1}). The same proportion between the concentrations of β-carotene, torularhodin, torulene, and γ-carotene (about 6:4:3:1) was determined along the subsequent days of fermentation. Therefore, the maximum concentration of β-carotene was reached at the fifth day (218 µg g^{-1}). β-Carotene is considered the most interesting carotenoid type, because it is the main safe dietary source of vitamin A and its antioxidant activity (Kot et al., 2016). As reported in the literature, the oxidation of carotenes to torulene and torularhodin is promoted by microbial cultures under oxidative conditions (Varmira et al., 2016; Zoz et al., 2015). Currently, torulene and torularhodin are not commercially used. However, it is known that torulene exhibits provitamin A properties and antioxidative action (Maldonade et al. 2008; Kot, et al., 2016). Thus, native *R. marina* strain may be the biocatalyst of choice for the conversion of the studied solid wastes.

Table 3. Specific carotenoid production rates for yeast cultures using different substrates.

SPC*	Yeast	Substrate	Reference
126	*R. glutinis*	Rectified grape must	Buzzini and Martini (2000)
118	*R. mucilaginosa* 137	YM broth	Maldonade et al., 2008
6.6	*R. minuta*	YM broth	Maldonade et al., 2008
14.4	*Sporobolomyces roseus*	YM broth	Maldonade et al., 2008
81.9	*Rhodosporidium paludigenum*	Glycerol, Urea	Yimyoo et al., 2011
124.3	*Sporidiobolus pararoseus*	Glycerol, corn steep liquor	Valduga et al., 2014
102.4	*R glutinis*	Cheese whey	Kanzy et al., 2015
151.6	*R mucilaginosa*	Cheese whey	Kanzy et al., 2015
54.8	*R mucilaginosa*	Cassava bagasse	Manimala and Murugesan, 2017
38.4	*R mucilaginosa*	Rice flour	Manimala and Murugesan, 2017
65	*Sporobolomyces ruberrimus*	Raw glycerol, fatty acids	Cardoso et al., 2016

* SPC is the specific carotenoid production rate ($\mu g_{carotenoids}$ g^{-1} day^{-1}).

Table 4. DAD UV-*vis* wavelengths of maximum absorbance and main APCI-MS peaks of *R. marina* cultivated in liquid medium containing raw glycerin, spent brewer's yeast, or urea.

Carotenoid	APCI m/z	MAW (nm)
Torulene	535.4 $[M+H]^+$	485
β –carotene	537.4 $[M+H]^+$	452
γ-carotene	537.4 $[M+H]^+$	459
Torularhodin	565.6 $[M+H]^+$	500

Maldonado et al. (2008) reported obtaining levels of torulene (38-54 μg g^{-1}), torulorradine (2.0-6.6 μg g^{-1}), and β-carotene (13-16 μg g^{-1}) in cultures of *R. mucilaginosa* strains. Yimyoo et al. (2011) reported cultures of *Rhodosporidium paludigenum* DMKU3-LPK4 with a yield of 0.31 mg g^{-1} carotenoids in 132 h of incubation. More recently, Andrade et al. (2016) demonstrated that *R. glutinis* strains presented the highest productivity of total carotenoids (128 μg g^{-1}) in medium containing raw glycerin. The yield for β-carotene was 82.02 μg g^{-1}, in the absence of illumination. Thus, a *R. marina* strain native to Brazil has the potential to add value to the tested wastes by substituting them for high-cost raw materials.

CONCLUSION

The results show that a strain of native *R. marina* from Brazil may be the biocatalyst of choice for the conversion of two solid wastes produced on an industrial scale (spent brewer's yeast and raw glycerin) to substitute primary carbon and nutrient sources in the production of value-added metabolites (β-carotene, torularhodin, torulene, and γ-carotene) and a means of valorization of solid wastes from the biodiesel and brewery industry.

NOMENCLATURE

ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionization
DAD	Diode array detector
HPLC	High-performance liquid chromatography
MAW	Maximum absorbance wavelength
$[M+H]^+$	Protonated molecular ion
MS	High-resolution mass spectrometer
SBY	Spent brewer's yeast
SCV	Superior calorific value
TCLP	Toxicity characteristic leaching procedure
UV-*vis*	Ultraviolet visible

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